

Saphenofemoral Venous Channels Associated with Recurrent Varicose Veins are not Neovascular

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Background. Recurrence of varicose veins after apparently adequate surgery is common. Neovascularisation, the formation of new vascular channels between a venous surgery site and new varicosities, is thought to be an important cause of recurrence. The aim of this study was to provide histological evidence of the 'neovascularisation' process.

Method. Tissue samples from the region of the previously ligated saphenofemoral junction (SFJ) were taken from 14 limbs with recurrent varicose veins and from nine control limbs. Tissue samples were analysed histologically for overall vascularity, and the presence of intimal circular fibrosis, intimal eccentric fibrosis, medial thickened elastosis, and thrombosis in the microscopic thin walled vessels within the tissue. The same samples were analysed immunohistologically for S100, a neural marker, and Ki-67 (Mib 1), a marker of endothelial proliferation. Absent S100 and positive Ki-67 were considered as evidence of new vessels.

Result. No significant difference was found between the venous recurrence and control groups in respect to histological features. S100 positive nerve fibrils were seen associated with dilated venous channels in the majority of both redo and control groups ($p=1$, Fisher's exact test). Only one section stained positively with Ki-67 (Mib1) in a single vascular channel for a few endothelial cells. The remaining control and redo cases were negative for Mib 1 ($p=1$, Fisher's exact test).

Conclusion. We found little evidence of neovascularisation associated with recurrent varicose veins in the saphenofemoral region. The venous channels that develop at the previously ligated SFJ may represent adaptive dilatation of pre-existing venous channels (vascular remodelling), probably in response to abnormal haemodynamic forces.

Keywords: Recurrent varicose veins; Neovascularisation; Histology.

Introduction

The recurrence of varicose veins following surgery is a common and costly problem despite current improvements in pre-operative evaluation and therapeutic interventions, and its prevalence varies among published reports from 20% to as high as 80%.^{1–6} For those requiring surgery, in the United Kingdom, varicose vein operations comprise both ordinary admissions and day cases total in excess of 60,000 per annum, with an almost equal number of hospital bed days being used.⁷ Therefore, even a recurrence rate of 20% will have considerable health economic impact.

Traditionally, it was thought that the most common

cause for recurrence is failure to perform adequate saphenofemoral junction (SFJ) ligation and stripping of the great saphenous vein (GSV) at the primary operation.^{8–12} However, there has been evidence showing recurrent varicose veins in which incomplete surgery is not obviously involved while development of new veins at the SFJ has been observed using duplex imaging examinations. This has led many investigators to postulate that these new vessels grow to join with an existing GSV or other residual thigh veins and have called this process as neovascularisation.^{4,6,13–15} Nevertheless, other investigators remain dubious about the development of neovascularisation as mechanism for recurrent varicose veins and postulate that pre-existing tributaries of the common femoral vein expand in response to SFJ ligation.¹⁶

Imaging methods such as varicography and duplex scanning provide indirect evidence^{6,9,12,14,15,17,18} and cannot prove directly the development of neovascularisation as mature neovascular veins may have

This paper has been awarded the 3rd Prize of the 5th Annual Meeting of the European Venous Forum, 25–27 June 2004, Warsaw, Poland.

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luminal appearances indistinguishable from collateral veins.

The only direct evidence for new vein growth in the literature depends upon a single paper, which failed to detect S100 protein, a neural marker associated with existing vascular structures, in the new veins.¹⁹ The authors interpreted this finding as indicating that these veins formed *de novo*, rather than from pre-existing venous channels.

This immunohistological study was conducted to investigate re-operative tissue for neovascular molecular markers in order to demonstrate the presence of neovascularisation in a series of patients with recurrent varicosities.

Methods and Materials

Fourteen limbs with recurrent varicose veins (venous recurrence group) of fourteen patients with a mean age of 57 years (range 33–69 years) and nine limbs (control group) of nine patients (mean age 49 years, range 45–52) subjected to an initial surgery for primary varicose veins or to surgery for recurrent inguinal hernia were included in the study. These patients represented all suitable patients operated in a three-month time frame. In limbs with venous recurrence the mean interval time between initial surgery and redo surgery was 11 years (range from 2 to 40 years).

All limbs prior to redo operation had a duplex scan by experienced technicians. The ultrasonographer noted the presence or absence of the SFJ, GSV, saphenopopliteal junction (SPJ) and small saphenous vein (SSV). Reflux at any of these sites or levels, along with deep vein and perforator incompetence was noted. The presence of small veins in the groin, or reconstitution of the SFJ with numerous serpentine small incompetent veins were specifically noted.

In all cases, that were included in the venous recurrence group, the ultrasonographer reported the presence of 'small veins' in the groin, 'reconstituted SFJ', or 'numerous incompetent vessels with turbulent flow' at the sites corresponding to the site of the previously ligated SFJ. These cases were interpreted to represent neovascularisation as a cause of recurrent varicose veins.

All operations were performed as day cases. In all limbs with vein recurrence the SFJ was re-explored by a consultant vascular surgeon. At operation the presence of serpentine vessels, an intact SFJ, a patent LSV, and major tributaries were noted.

Tissue samples from the SFJ stump were taken from all 14 limbs with recurrent venous disease as well as

from the nine control limbs (four primary saphenofemoral and two primary saphenopopliteal ligations, two inguinal hernias, and one sample of varicose veins which had been chronically occluded by thrombophlebitis). In redo operations, the incision used was oblique through the previous incision and the scar tissue towards the SFJ stump with the surrounding subcutaneous fat containing small venules was taken *en bloc* for histology. In all cases, a patient's informed consent was taken according to Ethical committee guidelines.

The samples were fixed in 10 percent buffered formalin and were subsequently processed and embedded in paraffin wax. The samples were sectioned at four microns onto glass slides coated with 3-aminopropyltriethoxysilane (APES). Two pathologists independently assessed histological features on hematoxylin and eosin stained slides; specifically the tissue overall vascularity, the presence of intimal circular fibrosis, intimal eccentric fibrosis, medial thickened elastosis which identified the vessels as venous rather than arterial, and thrombosis in the vessels of the tissue samples. These features were scored as absent (0), mild, moderate or severe (1–3). For fibrosis, mild is defined as less than 15% lumen loss, moderate up to 50% and severe >50%. Elastosis is a judgemental score reflecting a staining quality; mild represents a few elastic laminae replicated focally; moderate, general replication of 1–3 layers; and severe, multiple layers often with fibrosis and wall thickening.

The sections were also stained by standard immunochemistry protocols with antibodies to S100 (Dako) and Ki-67 (Mib 1) and examined for neural tissue and endothelial proliferation using the respective markers by the two pathologists independently in a blinded fashion. The results were again expressed as 0, 1, 2 or 3 score, corresponding to absent, mild, moderate or extensive staining. Where there was a difference in the scoring/analysis of the sections, the samples were reviewed by both pathologists simultaneously and a consensus derived.

Statistical analysis was made by the Fisher's exact test and statistical significance was reached when *p* was less than 0.05.

Results

No statistical significant difference was found between the venous recurrence and control groups in respect to all examined histological features (Table 1).

S100 positive nerve fibrils were seen within the majority of dilated vascular channels in most of the

Table 1. Scoring of histological and immunohistological sections

Case	Overall vascularity	Intimal circular fibrosis	Intimal eccentric fibrosis	Media thickened elastosis	Thrombosis	Mib-1 staining	S100 staining
Recurrent VVs							
1	0	0	1	1e	0	0	1
2	2	1	1	2.	0	0	2
3	2	2	0	2.	0	0	1
4	0	1	1	1ee	0	0	1
5	1	1	1	1e	0	0	1
6	1	0	0	0.	0	0	0
7	0	1	2	2.	0	0	0
8	0	0	2	0.	0	0	1
9	1	1	1	1e	0	0	1
10	1	1	2	1e	0	0	1
11	1	0	1	1.	0	0	1
12	0	1	0	1.	0	0	0
13	0	0	1	1e	0	0	1
14	0	1	2	1e	0	1	0
Controls							
LSV	1	0	1	1.	0	0	1
LSV		1	2	2e	0	0	0
LSV	1	1	2	1e	0	0	1
SFJ		0	2	3e	0	0	0
SPJ		0	2	1e	0	0	0
SPJ	0	1	1	0.	0	0	1
Minor VV	0	0	1	1.	0	0	1
RIH	1	1	3	1e	0	0	1
RIH	2	0	0	0.	0	0	1

p, all recurrent VV samples were from the saphenofemoral junction area. Control samples comprised; LSV, long saphenous vein from first time operations; SFJ, SPJ, tissue from saphenofemoral and saphenopopliteal junction areas from first time operations; Minor VV, calf and thigh varicosities occluded by thrombophlebitis; RIH, recurrent inguinal hernia tissue.

cases in both the redo and control groups (Table 1, Fig. 1). S100 staining was noted to be absent in 4 out of 14 recurrent varicose vein samples and in 3 out of 9 control sites. Only one section stained positively with Ki-67 (Mib1) in a single vascular channel for a few endothelial cells. The remaining control and redo cases were negative for Mib 1 (Fig. 2). There was positive Ki-67 staining in lymphoid foci found in some of these

groin tissues, representing a positive control for the staining process.

Discussion

Neovascularisation has been considered as an important cause of recurrent varicose veins in a considerable number of recent publications.^{4,6,13–15,17} Neovascularisation was defined as the presence of multiple

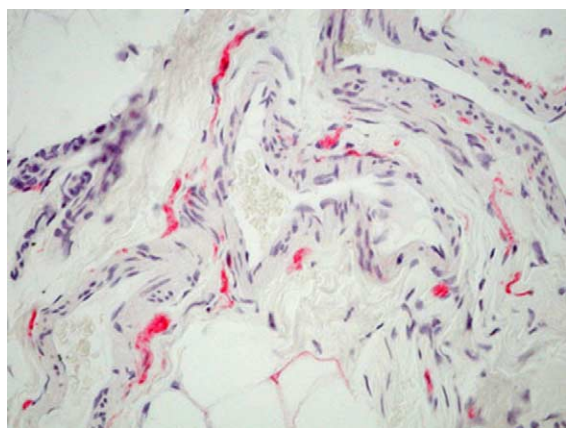


Fig. 1. Convoluted small vessels are seen with S100 positive nerve fibrils locally (Alkaline Phosphatase technique; S100 positive staining is shown in pink, original magnification $\times 400$).

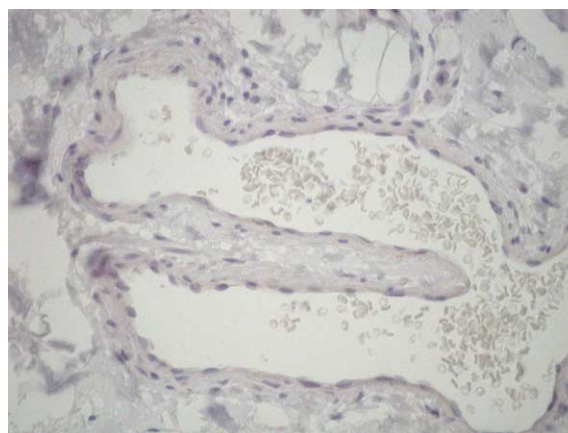


Fig. 2. Vascular endothelial cells are negative for Mib-1 antibody staining (original magnification $\times 400$).

small tortuous connections between the saphenous stump on the femoral vein and the residual saphenous vein or its tributaries.^{9,20,21} Duplex scanning and varicography has been used in arriving at these conclusions.^{6,12,14,15,17,18,22} However, imaging methods such as varicography and duplex scanning cannot prove whether these small venous channels are truly neovascular, or derive from expansion of existing venous collaterals.

The existence of neovascularisation process has been initially demonstrated in animal studies, firstly in the rabbit ear chamber model^{23–25} and secondly, after ligation of the rat femoral vein.²⁶ It has also demonstrated by means of phlebography, surgical assessment and histology of excised tissue in patients with varicose veins after transection of the GSV in the lower part of the thigh.²⁷

The concept of neoangiogenesis, new vessels growing as capillary buds proliferating into adjacent tissues, has prompted other groups to use S100 immunohistochemistry as a marker of neovascularisation. Vessels are generally accompanied by nerve fibrils, which highly express S100 protein, particularly S100A1 and S100B,²⁸ but true new vessels in granulation tissue and tumour circulations are not thought to be accompanied by new nerve growth.¹⁹ Previous work by Nyamekye *et al.*, supported neovascularisation in cases of recurrent varicose veins by demonstrating an absence of S100 positive neural tissue associated with venous channels.¹⁹

In the present study, it was demonstrated that there was a high incidence of S100 positive tissue within vessel walls in both control and recurrence groups, which contradict the results of the latter report.¹⁹

Mib1 is a monoclonal antibody that recognizes proliferating cells by binding to Ki-67. It has been used as a proliferative marker in a wide spectrum of neoplastic and non-neoplastic conditions.²⁹ It identifies a nuclear antigen associated with the cell cycle, being expressed in all phases except G0, where the cell cycle is in a resting state. Ki-67 positivity informs us that tissue proliferation has taken place, but the important question of when that proliferation occurred remains to be answered. It is possible that as samples were gathered many years after initial surgery, that any proliferative event is far in the past, and not detectable by tissue sampling at re-operation. However, this study did not detect markers of endothelial cell proliferation consistent with a neovascular theory. In our study, Mib-1 staining was not present in any of the control samples and in all but one of the recurrence group samples.

In respect to the overall vascularity, vessel intimal circular fibrosis, intimal eccentric fibrosis, media

thickened elastosis and thrombosis no differences were observed in the histological analysis of the samples between the control and the venous recurrence groups. All these again can be interpreted as evidence of vessels with characteristics of collaterals found in human tissue but without the presence of neovascular growth.

Certainly there is discrepancy in results between our work and the work of others.¹⁹ Immunostaining is not an exact science. Nyamekye *et al.*¹⁹ used positive controls for S100 staining from other sections of breast and groin tissue, but could not detect S100 positivity in their redo groin tissues. There may be many reasons why we found S100 positive 'neovessels' and they did not; all our staining was performed in a single batch, and although we assume that was the case with Nyamekye *et al.*, it is not specifically stated in their article. Their antibody source was different to ours, and may have had different specificities for different S100 proteins. Possibly, as time passes, S100 negative neovessels undergo neural ingrowth and become S100 positive; this seems unlikely, and contradicts the premise of Nyamekye's investigation. The interval between initial and recurrent vein surgery in our group (median 11 years, range 2–40 years) was not longer than in Nyamekye's study (median 11.5, range 3–46 years) so there was no difference in time scale for hypothetical nerve ingrowth to occur. Negative staining results may be obtained for various reasons, which may be related to the altered sensitivity of used antibodies to S100. Therefore, caution is required when negative results are used as definite evidence for or against an hypothesis; for negative evidence is overturned by even a single positive finding. However, when there is positive evidence from positive S100 staining the results are considered reliable and thus conclusions can be drawn.^{30,31}

It has never been satisfactorily explained why new vessels should grow towards a target vein, be it a thigh varicosity or a persistent GSV. Arterial neovascular circulations in tumours are notoriously disorganised, even in the presence of clear chemotactic, angiogenic and vasculogenic influences from the tumour.³²

As an interpretation of our findings we can postulate that they are indicative of the presence of venous channels that are expansions of pre-formed collaterals, rather than new vessel growth.¹⁶ We consider that the directional nature of the small veins seen in the groin are collateral channels opening up in the presence of abnormal haemodynamic stimuli, most likely a type of pulling force from the persistent LSV, acting as a sump drain, but possibly also from a pushing type of force from persistent deep venous incompetence. Additionally, factors that were

involved with the original varicose veins, such as venous wall distensibility, and valvular weakness, are still present and it is reasonable to postulate that these are involved with the recurrence as well. The important role of these factors can be appreciated by the fact that these phenomena do not occur when SFJ is ligated for GSV harvesting in femoropopliteal bypass, suggesting that it develops uniquely in the lower limb varicose vein disease.

Finally, our present work concludes that neovascular vessels are not more common in saphenofemoral recurrent varicose veins than in other post-surgical scar tissue. Our findings challenge the concept that new vessel growth is responsible for the formation of multiple serpentine veins in the groin. Thus, we hypothesise that altered venous haemodynamic forces may cause remodelling of pre-existing minor venous collaterals.

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Accepted 13 September 2004

Available online 12 October 2004